A NEW METHOD OF SEPARATING FUNGI FROM PROTOZOA AND BACTERIA

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The investigations of RUSSELL and HUTCHINSON,[1] GOODEY,[2] CUNNINGHAM and LÖHNIS,[3] and others have stimulated the development of a new branch of soil biology, namely, soil protozoology. A problem of considerable interest in this field is the determination of the effect of soil protozoa upon soil bacteria. RUSSELL and HUTCHINSON maintain that soil protozoa have a marked influence on bacterial activity, and consequently soil protozoa may be regarded as one of the limiting factors in soil fertility. The criterion for measuring the effect of soil protozoa on bacterial activity has commonly been the production of ammonia and nitrates.

In pursuing this problem, some preliminary experiments, one of which was concerned with soil fungi, were carried on by the writers. In view of the fact that fungi are capable of producing ammonia,[4] their presence might mean an additional factor not accounted for in measuring the effect of soil protozoa on soil bacteria. So far as we have been able to ascertain, neither RUSSELL and HUTCHINSON nor other investigators dealing with similar problems have taken into consideration the possible value of this factor. Fungi are capable of producing ammonia, and until it has been established that they do not alter the results of ammonia production in the presence of bacteria in such experimental work, it would seem a priori that their presence is undesirable. Since a survey of the literature bearing on the subject offered neither suggestions nor a solution of the difficulty, the authors attempted to devise a method for the elimination of this factor.

[1] RUSSELL and HUTCHINSON, Jour. Agric. Sci. **3**:111. 1909; **5**:152. 1913.

[2] GOODEY, Proc. Roy. Sci. London **84**:165. 1911.

[3] CUNNINGHAM and LÖHNIS, Centralbl. f. Bakt. II. **39**:596. 1913; **42**:8. 1914.

[4] MÜNTZ and COUDON, Compt. Rend. **116**:395. 1893.
MARCHAL, Bull. Acad. Sci. Belg. III. **25**:727. 1893.
MCLEAN and WILSON, N.J. Bull. 270. 1915.

The method finally devised and tested is based upon the principle of dilution, in such a manner as to reduce the possibilities for the occurrence of fungi in the cultures. Poured agar plates were used for this purpose. The method of procedure was as follows:

SERIES 1.—Plates of 10 different agar media suitable for growing fungi were poured in duplicate. They were potato, oat, cornmeal, rice, bean, raisin, apple, synthetic (LIPMAN and BROWN, N.J. Ann. Rep. 1908. p. 133), soil extract agar (prepared by adding 15 gm. agar to 1000 cc. Löhnis soil extract), and Cook's fungi medium no. II (Del. Bull. 91. p. 10).

After cooling, a block of each medium about 2 cm. square was cut out with a sterile knife, and 1 cc. of sterile soil extract was introduced by means of a sterile pipette into the cavity formed. A platinum loopful of a 3-day old culture of soil organisms in soil extract, known to contain numerous bacteria, protozoa, and fungi, was then carefully rinsed off in the medium. This soil extract was prepared according to Löhnis' directions by heating 1 kg. of good soil with 1 liter of water at 15 lbs. pressure in the autoclave half an hour. It was then removed, mixed with a generous quantity of talc, shaken thoroughly, and the liquid filtered through a double thickness of filter paper, until a clear solution was obtained. The moist residue of soil in the flask was pressed out to remove any solution still remaining. The solution was made up to a volume of 800 cc. and 0.05 per cent K_2HPO_4 added.

SERIES 2.—This served as a check on series 1, consisting of poured plates each inoculated with one loopful of the same 3-day old culture of organisms, and made at the same time, using the 10 different media previously mentioned.

SERIES 3.—After one week, poured plate cultures were made, using the same media and inoculating with one loopful of the solution taken from the cavities of the agar plates of series 1, in order to make doubly certain that fungi were not present.

The results in series 1 show that on the plates where a portion of the agar was removed and 1 cc. of soil extract substituted, the bacteria and protozoa developed in enormous numbers, which might be due to the fact that a large surface is exposed for such a relatively small quantity of media. The important point, however,

which is to be noted from this experiment, is that despite the fact that media were furnished for the growth of fungi none was evident, even after 30 days' incubation.

From the observation of the poured plate cultures of series 2, made from the same 3-day old culture as series 1, it was noted that fungi appeared after 4 days upon 3 out of 10 plates; namely, Cook's no. II, Lipman and Brown's synthetic, and raisin agar. The predominating fungi were species of *Penicillium*, *Alternaria*, and *Fusarium*. On the poured plate cultures of series 3, inoculated with the solution in the cavities of the agar plates in series 1, no fungi developed. This experiment was repeated and corroborated the previous results. Thus it appears that although fungi were present in the original culture, the process of high dilution was responsible for their elimination in the specially prepared cavity on the agar plates in series 1.

Another method with the same object in view, namely, the separation of fungi from bacteria and protozoa, was employed, the procedure of which was as follows: Poured plate cultures of the 10 different media (as before) were made from the same 3-day old culture of soil extract known to contain numbers of bacteria, protozoa, and fungi, and the plates were watched carefully throughout the period of one week's incubation for the appearance of any fungi. As soon as their presence was discerned, the fungous growths were removed with a sterile scalpel. At the end of one week, at which time it was reasonably certain that the fungi had ample opportunity to develop, a portion of the agar, about 1 sq. in. in size, was removed with a sterile scalpel from each of the 10 plates, placed in 50 cc. of soil extract, and the flask thoroughly shaken to disintegrate the agar. For 4 days a microscopic observation was made and a few small flagellates and small ciliates were discovered on the preparation from raisin agar, and a few small ciliates and numerous small flagellates on the apple agar. No large ciliates were noted.

Summary

1. The dilution method followed by the peculiar manner of plating as outlined makes it possible to separate fungi from bacteria and protozoa.

2. As a result of this separation it is possible to eliminate fungi from experiments involving the effect of protozoa upon bacterial activity, by making a subculture from the fungi-free solution of bacteria and protozoa (in the cavity of the agar plate).

3. The second method described, that of removing the fungi from the plates as they appeared, is undesirable for our special investigation, for the reason that the bacteria are allowed to multiply easily, while the protozoa have no such favorable conditions; consequently, on transferring such a culture to the soil, the protozoa would be at a considerable disadvantage, and their activity would be seriously inhibited if not entirely suppressed. The suggestion may be offered, however, that this method might be employed for obtaining cultures of single types of protozoa, as for example small flagellates or small ciliates.

This paper represents one phase of the preliminary work undertaken in connection with an investigation of the effect of soil protozoa upon the activity of soil bacteria. Further results on experimentation and a bibliography on soil protozoa and soil sterilization are awaiting publication.

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